TECHNICAL BULLETIN

GoTaq® PCR Core System

Instructions for Use of Product
M7660

Revised 5/19
TB254

GoTaq® PCR Core System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The GoTaq® PCR Core System I[a,b] provides all of the reagents necessary for the exponential amplification of specific regions of DNA using the polymerase chain reaction (PCR; 1). The GoTaq® PCR Core System I is supplied with GoTaq® DNA Polymerase, a proprietary formulation of *Taq* DNA polymerase designed for enhanced amplification, and GoTaq® Flexi Buffers, magnesium-free proprietary buffer formulations supplied at pH 8.5. The 5X Green GoTaq® Flexi Buffer increases the density of the sample and contains two dyes (a blue dye and a yellow dye) that separate during electrophoresis, allowing reactions to be loaded directly onto an agarose gel without the addition of loading dye. During electrophoresis in a 1% agarose gel, the blue dye migrates at the same rate as a 3–5kb DNA fragment, and the yellow dye migrates slightly faster than primers (<50bp). The 5X Colorless GoTaq® Flexi buffer allows fluorescence or absorbance measurements to be made directly on the amplified DNA without the need to purify it from the amplification reaction.

Although simple in theory, PCR can benefit from optimization of several parameters. This technical bulletin provides important and convenient information about performing PCR and includes protocols and troubleshooting tips for successful PCR amplifications.

Comprehensive information on the PCR process is available online in the PCR Chapter of the *Protocols and Applications Guide* at: **www.promega.com/paguide**

2. Product Components and Storage Conditions

PRODUCT	CAT.#
GoTaq® PCR Core System I	**M7660**

For Laboratory Use. Includes:

- 250u GoTaq® DNA Polymerase
- 2 × 1ml 5X Colorless GoTaq® Flexi Buffer, Mg-Free
- 2 × 1ml 5X Green GoTaq® Flexi Buffer, Mg-Free
- 1.2ml $MgCl_2$, 25mM
- 200µl PCR Nucleotide Mix, 10mM

Storage Conditions: Store all components at –20°C. See product label for expiration date.

3. PCR Protocol

To facilitate optimization, troubleshooting and validation of PCR, we strongly recommend performing concurrent positive and negative control reactions.

If working with multiple samples, assemble a master mix of water, $MgCl_2$, 5X buffer, primers, PCR Nucleotide Mix and GoTaq® DNA Polymerase. Combine the appropriate multiples of these components (except template) and dispense aliquots of the appropriate volume, minus the specific volume to be occupied by the template DNA, to each reaction tube. Initiate the reaction by adding the template. Use individual pipette tips for all additions, being careful not to cross-contaminate the samples.

If possible, start with $>10^4$ copies of the target sequence to obtain a signal in 25–30 cycles. Excess template is not beneficial to the reaction. Always ensure that the final DNA concentration is ≤10ng/µl. Less than 10 copies of a target can be amplified (2), but more cycles may be required to detect a signal by gel electrophoresis. Additional cycles may increase nonspecific amplification, evidenced by multiple bands when analyzed by gel electrophoresis.

Materials to Be Supplied by the User

- Mineral Oil
- Nuclease-Free Water (Cat.# P1193)
- template DNA
- upstream primer
- downstream primer

3.A. Protocol

1. Combine the components, as listed in Table 1, in sterile, 0.5–0.6ml microcentrifuge tubes on ice. Amplification reactions may be scaled up or down as necessary. Use of a master mix, as described above, greatly facilitates the reaction setup and decreases tube-to-tube variability of the reaction components. Thaw the 25mM $MgCl_2$ solution and GoTaq® Flexi Buffer completely and vortex thoroughly before use. We strongly advise optimizing the $MgCl_2$ concentration.
2. If using a thermal cycler without a heated lid, overlay the reaction mix with 1–2 drops (approximately 50µl) of Mineral Oil to prevent evaporation during thermal cycling. Centrifuge the reaction mix in a microcentrifuge for 5 seconds.
3. Place the reactions in a thermal cycler that has been preheated to 95°C. We recommend heating the samples at 95°C for 2 minutes to ensure that the target DNA is completely denatured. Incubation longer than 2 minutes at 95°C is unnecessary and may reduce the yield.
4. Start the thermal cycling program. The cycling profile given in Table 2 may be used as a guideline. We recommend that you optimize the amplification profile for each primer/target combination.

3.A. Protocol (continued)

Table 1. Recommended Reaction Volumes and Final Concentrations of the GoTaq® PCR Core System Components.

Component	Component Volume (25µl)	Component Volume (50µl)	Final Concentration
$MgCl_2$, 25mM Solution (see Section 4.B)	1.0–4.0µl	2.0–8.0µl	1.0–4.0mM
5X Colorless GoTaq® Flexi Buffer OR 5X Green GoTaq® Flexi Buffer	5µl	10µl	1.0X
PCR Nucleotide Mix, 10mM each	0.5µl	1µl	200µM each
upstream primer	2.5–25pmol	5–50pmol	0.1–1.0µM
downstream primer	2.5–25pmol	5–50pmol	0.1–1.0µM
GoTaq® DNA Polymerase, 5u/µl	0.125µl	0.25µl	0.625u/25µl 1.25u/50µl
template DNA	variable	variable	<0.25µg/25µl <0.5µg/50µl
Nuclease-Free Water to a final volume of	**25µl**	**50µl**	

Thaw the 25mM $MgCl_2$ solution and GoTaq® Flexi Buffer completely and vortex thoroughly before use. We strongly advise optimizing the $MgCl_2$ concentration.

Table 2. Thermal Cycling Guidelines for PCR Amplification.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5–1 minute	25–35 cycles
Annealing	42–65°C*	0.5–1 minute	
Extension	72°C	1 minutes/kb	
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	indefinite	1 cycle

These guidelines apply to target sequences between 200–2,000bp and may need to be adapted for your thermal cycler.

*The annealing temperature for a specific amplification reaction will depend upon the sequences of the two primers. See Section 4.G for discussions on how to determine optimal annealing temperatures for PCR amplification.

3.B. Analysis

1. Analyze PCR products by agarose gel electrophoresis. The products should be readily visible in an ethidium bromide-stained gel under UV light. Reactions containing Green GoTaq® Buffer do not need loading dye added before electrophoresis.
2. Store PCR products at –20°C until needed. The PCR products can be further purified using a number of procedures including the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).

4. Optimization of PCR

4.A. Routine PCR

As originally developed, the PCR process amplifies short (approximately 100–500bp) segments of a longer DNA molecule (1). A typical amplification reaction includes the target DNA, a thermostable DNA polymerase, two oligonucleotide primers, deoxynucleotide triphosphates (dNTPs), reaction buffer, magnesium and optional additives. The components of the reaction are mixed and placed in a thermal cycler, an automated instrument that 'cycles' the reaction through a predetermined series of specific temperatures and times. One cycle of amplification is defined by the series of temperature and time adjustments. Each cycle of PCR after the first cycle theoretically doubles the amount of targeted template sequence (amplimer). Therefore, ten cycles theoretically multiplies the amplimer by a factor of about one thousand; 20 cycles, by a factor of more than one million. PCR amplification can be completed in as little as 2 hours.

Each cycle of PCR amplification consists of a defined number of reaction steps. The steps are designed using temperature and duration time to denature the template, anneal the two oligonucleotide primers and extend the new complementary DNA strands by polymerization. These steps can be optimized for each template and primer pair combination. The target DNA is denatured by heating to 95°C or higher for 15–120 seconds. During denaturation, the two intertwined strands of DNA separate from one another, producing the necessary single-stranded DNA (ssDNA) template for primer annealing and polymerization (extension) by a thermostable polymerase. To anneal the oligonucleotide primers, the temperature of the next step in the cycle is reduced to approximately 42–65°C. At this temperature, the oligonucleotide primers can anneal to the ssDNA strands and serve as primers for DNA synthesis by the polymerase. This step requires approximately 30–60 seconds. Finally, to extend from the primer-bound template DNA, the reaction temperature is raised to the optimum for most thermostable DNA polymerases, which is approximately 72°C. Extension of the primer by the thermostable polymerase requires approximately 1 minute per kilobase to be amplified. Extension completes one cycle, and the next cycle begins by returning the reaction to 95°C for denaturation. After 20–40 cycles, the amplified nucleic acid may then be analyzed (e.g., for size, quantity or sequence) or it may be used in further experimental procedures (e.g., cloning or mutagenesis).

4.B. Magnesium Concentration

Magnesium concentration is a crucial factor affecting the performance of thermostable enzymes. Reaction components, including template DNA, chelating agents present in the sample (e.g., EDTA or citrate), dNTPs and proteins, can affect the amount of free magnesium. In the absence of adequate free magnesium, GoTaq® DNA Polymerase is inactive. Conversely, excess free magnesium reduces enzyme fidelity (3) and may increase the level of nonspecific amplification (4,5). For these reasons, it is important to empirically determine the optimal $MgCl_2$ concentration for each reaction. This is accomplished by preparing a series of reactions containing 1.0–4.0mM Mg^{2+}, in increments of 0.5mM, by adding 2, 3, 4, 5, 6, 7 and 8µl of a 25mM $MgCl_2$ stock to 50µl reactions.

The GoTaq® PCR Core System includes 5X Green GoTaq® Flexi Buffer, Mg-Free; 5X Colorless GoTaq® Flexi Buffer, Mg-Free; and a solution of 25mM $MgCl_2$. The buffers allow you to adjust the Mg^{2+} concentration to the level that is optimal for each reaction using the 25mM $MgCl_2$.

Two important steps will ensure the reaction contains the appropriate amount of Mg^{2+}: Magnesium solutions must be thawed completely and vortexed for several seconds prior to use because magnesium chloride solutions can form concentration gradients when frozen. Thawing and vortexing is required to render the solution uniform with respect to magnesium salts. These two simple steps can eliminate a major source of many failed experiments.

4.C. Buffer Considerations

We recommend using the 5X Green GoTaq® Flexi Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium bromide staining. The 5X Green GoTaq® Flexi Buffer is not recommended for any downstream applications using absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications. The dyes absorb between 225–300nm, making standard A_{260} readings to determine DNA concentration unreliable. Also, the dyes have excitation peaks at 488nm and between 600–700nm that correspond to the excitation wavelengths commonly used in fluorescence detection instrumentation. However, for some instrumentation, such as a fluorescent gel scanner that uses a 488nm excitation wavelength, there will be minimal interference, since it is the yellow dye that absorbs this wavelength. Gels scanned by this method will have a light gray dye front below the primers corresponding to the yellow dye front. The Green and Colorless GoTaq® Flexi Buffers give approximately equivalent amplification yields. Balanced amplifications between the two buffers may require further optimization.

For reactions going directly from a thermal cycler to an application using absorbance or fluorescence, the 5X Colorless GoTaq® Flexi Buffer is recommended. If both agarose gel analysis and further downstream applications involving absorbance or fluorescence will be used, the two dyes can be removed from the Green GoTaq® Flexi reactions using standard PCR cleanup systems like the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or the Wizard® SV 96 PCR Clean-Up System (Cat.# A9341).

Both reaction buffers are compatible with common PCR additives such as DMSO and betaine. These additives do not change the color of the Green GoTaq® Flexi Buffer or affect dye migration.

4.D. Enzyme Concentration

We recommend that 1.25 units of GoTaq® DNA Polymerase be used per 50µl amplification reaction. For most applications, enzyme will be in excess; the inclusion of more enzyme will not significantly increase product yield. Increased amounts of enzyme and excessively long extension times increase the likelihood of generating artifacts. Artifacts generally can be seen as smearing of bands in ethidium bromide-stained agarose gels (6–8).

The most frequent cause of excessive enzyme levels is pipetting error. Accurate dispensing of submicroliter (<1µl) volumes of enzyme solutions in 50% glycerol is nearly impossible. We strongly recommend the use of reaction master mixes sufficient for the number of reactions being performed to obviate this problem. A master mix increases the volumes of pipetted reagents and reduces pipetting errors.

4.E. Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60% (G + C), and care should be taken to avoid sequences that might produce internal secondary structure. To avoid the production of primer-dimers, the 3´ ends of the primers should not be complementary. Primer dimers unnecessarily deplete primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3´ end of the primer, as this may result in nonspecific primer annealing, increasing the synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (T_m); in this manner, the two primers should anneal roughly at the same temperature. In any case, the annealing temperature of the reaction is dependent upon the primer with the lowest T_m.

The sequence of the primers can also include regions at the 5´ ends that may prove useful for downstream applications. For example, restriction enzyme sites can be designed into the primer pair for ease in downstream manipulations such as cloning. Regardless of primer choice, the final concentration of the primer in the reaction must be optimized. We recommend adding 50pmol of each primer (1µM final concentration in a 50µl reaction) as a starting point for optimization. Generally, nanograms of primer DNA equivalent to 50pmol is: 16.3ng × b, where b is the number of bases in the primer.

4.F. Template Considerations

Successful amplification of the region of interest depends upon the amount and quality of the template DNA. Reagents commonly used to purify nucleic acids (e.g., salts, guanidine, proteases, organic solvents and SDS) are potent inhibitors of DNA polymerases. A final ethanol precipitation of the nucleic acid sample will eliminate most of the inhibitory agents. Spiking a control DNA fragment and the appropriate primer pair into the DNA preparation may be useful in verifying the purity of the DNA sample.

The amount of template required for successful amplification depends upon the complexity of the DNA sample. For example, whereas a 4kb plasmid containing a 1kb insert equates to 25% of the DNA being the target of interest, a 1kb gene in human genomic DNA (genome of 3.3 × 109bp) represents approximately 0.00003% of the input DNA. Therefore, approximately 1,000,000-fold more human genomic DNA is required to maintain the same number of target copies per reaction.

4.F. Template Considerations (continued)

Two common mistakes encountered when trying to amplify target DNA are using too much plasmid DNA and too little genomic DNA. Table 3 lists the correlation of molecules per microgram of nucleic acids from some common RNA and DNA targets. Table 4 shows typical yields of genomic DNA from a variety of source materials. As a general guide for how much template DNA to use, start with a minimum of 10^4 copies of the target sequence to obtain a signal in 25–30 cycles, but keep the final DNA concentration of the reaction ≤10ng/µl.

Table 3. Conversion of Nucleic Acids from Microgram Amount to Number of Molecules.

Nucleic Acid	Amount	# of Molecules
1kb RNA	1µg	1.8×10^{12}
1kb dsDNA	1µg	9.18×10^{11}
pGEM® Vector DNA	1µg	2.9×10^{11}
lambda (λ) DNA	1µg	1.9×10^{10}
E. coli genomic DNA	1µg	2×10^{8}
human genomic DNA	1µg	3.0×10^{5}

Table 4. DNA Yields from Different Human Tissue Sources.

Amount of Material	Source of DNA Typically Used	Typical Yield
Whole blood	30µl	0.5–1µg
Blood spot	1/2 of a 5mm spot	1–3µg
Cell suspension	5×10^5 cells	2–5µg
Buccal cells	Single mouth rinse	0.1–1µg
Chorionic villus biopsy	Small frond	1–3µg
Semen	30µl	5–10µg
Hair root	Single root	10–200ng
Tissue block	50mg	0.1–10µg

4.G. Primer Annealing Temperature

The sequences of the primers are a major consideration in determining the optimal temperature of the PCR amplification cycles. For primers with a high T_m, it may be advantageous to increase the annealing temperature. Higher temperatures minimize nonspecific primer annealing, increase the amount of specific product produced and reduce the amount of primer-dimer formation.

Numerous formulas exist to determine the theoretical T_m of nucleic acids (9,10), and these may serve as a starting point for annealing conditions. However, it is best to optimize the annealing conditions by performing the reaction at several temperatures starting approximately 5°C below the calculated T_m.

For a T_m calculator that can calculate melting temperatures for pimers in a GoTaq® reaction, visit our web site at: **www.promega.com/biomath/**

The formula below can be used to estimate the melting temperature for any oligonucleotide:

$$T_m = 81.5 + 16.6 \times (\log_{10}[Na^+]) + 0.41 \times (\%G+C) - 675/n,$$

where $[Na^+]$ is the molar concentration of monovalent cations and
n = number of bases in the oligonucleotide.

Example: To calculate the melting temperature of a 22mer oligonucleotide with 60% G+C in 50mM KCl:

$$T_m = 81.5 + 16.6 \times (\log_{10}[0.05]) + 0.41 \times (60) - 675/22$$
$$= 81.5 + 16.6 \times (-1.30) + 24.60 - 30.68$$
$$= 53.84°C$$

4.H. Extension Temperature

During the extension step, allow approximately 1 minute for every 1kb to be amplified (minimum extension time of 1 minute). Generally, 25–40 cycles are sufficient for most reactions.

4.I. Nucleic Acid Cross-Contamination

It is important to minimize cross-contamination between samples and prevent carryover of RNA and DNA from one experiment to the next. Use separate work areas and pipettors for pre- and post-amplification steps. Use positive displacement pipettes or aerosol-resistant tips to reduce cross-contamination during pipetting. Wear gloves and change them often. Consider using a contamination control technique (11) to prevent DNA carryover to subsequent reactions.

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Low yield or no amplification	Insufficient number of cycles. Return reactions to thermal cycler for 5 or more cycles.
	Template degraded. Verify the integrity of the DNA by electrophoresis after incubation in the presence of Mg^{2+}.
	Thermal cycler programmed incorrectly. Verify that times and temperatures are correct. Use step cycles, not hold segments.
	Temperature too low in some positions of thermal cycler. Perform a set of control reactions to determine whether certain positions in the thermal cycler give low yields.
	Top of thermal cycler is open. The top must be closed for correct heating and cooling.
	Inhibitor present in DNA template. Reduce the volume of sample in the reaction. Ethanol precipitate template DNA to remove inhibitor.
	Improper reaction conditions. Reduce the annealing temperature and/or allow longer extension times for longer amplifiers. Also, optimize Mg^{2+} concentration.
	Missing reaction component. Check the reaction components and repeat the reaction.
	Mineral oil problem. The reaction must be overlaid with high-quality, nuclease-free light mineral oil. **Do not use autoclaved mineral oil.**
	Poor primer design. Make sure primers are not self-complementary or complementary to each other.
	Incorrect primer specificity. Verify that the primers are complementary to the appropriate strands.
	Primer concentration too low. Verify primer concentration in the reaction and increase the concentration as needed.
	Suboptimal reaction conditions. Optimize Mg^{2+} concentration, annealing temperature and extension time. Always vortex the Mg^{2+}. Verify that primers are present in equal concentrations.

Symptoms	Causes and Comments
Low yield or no amplification (continued)	Degraded nucleotides or primers. Keep nucleotides and primers frozen in aliquots, thaw quickly and keep on ice once thawed. Avoid multiple freeze-thaw cycles.
	Target sequence not present in target DNA. Redesign experiment or try other sources of target DNA.
Low yield or no amplification in mouse tail genotyping applications	Adjust annealing temperature. Reaction buffer composition affects the melting properties of DNA. See the BioMath Calculator to calculate the melting temperature for primers in the GoTaq® reaction (**www.promega.com/biomath**). The salt-adjusted and base-stacking T_ms are lower in GoTaq® reaction buffer than in *Taq* DNA polymerase reaction buffer.
	Reduce volume of template DNA in reaction or dilute template DNA prior to adding to reaction. Many DNA preparations for genotyping applications result in the copurification of amplification inhibitors. Reducing the amount of inhibitors present in the reaction may improve results. Diluting samples even 1:10,000 has been shown to be effective, depending on initial DNA concentration.
	Ethanol precipitate template DNA. Include an ethanol precipitation and wash step prior to amplification to remove inhibitors that copurified with the DNA.
	Add PCR enhancers. Adding PCR-enhancing agents may improve yields. General stabilizing agents such as BSA (Sigma Cat.# A7030; final concentration 0.16mg/ml) may also help to overcome amplification failure.
Multiple, nonspecific amplification products	Suboptimal reaction conditions. Optimize $MgCl_2$ concentration, annealing temperature, size, extension time and cycle number to minimize nonspecific priming.
	Poor primer design. Make sure primers are not self-complementary or complementary to each other, especially near the 3´ ends. Try a longer primer. Avoid use of three consecutive G or C nucleotides at the 3´ end of the primers.
	Primer concentration too high. Verify primer concentration in the reaction. Try a lower concentration in the reaction.

5. Troubleshooting (continued)

Symptoms	Causes and Comments
Multiple, nonspecific amplification products (continued)	Contamination by another target DNA. • Use positive displacement pipettes or aerosol-resistant tips to reduce cross-contamination during pipetting. • Use a separate work area and pipettor for pre- and post-amplification. • Wear gloves and change them often. • Use UNG (11) or another contamination control technique to prevent DNA carryover to subsequent reactions.
	Multiple target sequences exist in target DNA. Design new primers with higher specificity to target sequence.

6. References

1. Saiki, R. *et al.* (1985) Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**, 1350–4.
2. Saiki, R.K. *et al.* (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–91.
3. Eckert, K.A. and Kunkel, T.A. (1990) High fidelity DNA synthesis by the *Thermus aquaticus* DNA polymerase. *Nucleic Acids Res.* **18**, 3739–44.
4. Williams, J.F. (1989) Optimization strategies for the polymerase chain reaction. *BioTechniques* **7**, 762–9.
5. Ellsworth, D.L., Rittenhouse, K.D. and Honeycutt, R.L. (1993) Artifactual variation in randomly amplified polymorphic DNA banding patterns. *BioTechniques* **14**, 214–7.
6. Longley, M.J., Bennett, S.E. and Mosbaugh, D.W. (1990) Characterization of the 5´ to 3´ exonuclease associated with *Thermus aquaticus* DNA polymerase. *Nucleic Acids Res.* **18**, 7317–22.
7. Sardelli, A.D. (1993) Plateau effect—understanding PCR limitations. *Amplifications* **9**, 1–6.
8. Bell, D.A. and DeMarini, D.M. (1991) Excessive cycling converts PCR products to random-length higher molecular weight fragments. *Nucleic Acids Res.* **19**, 5079.
9. Baldino, F., Chesselet, M.F. and Lewis, M.E. (1989) High-resolution in situ hybridization histochemistry. *Methods Enzymol.* **168**, 761–77.
10. Rychlik, W., Spencer, W.J. and Rhoads, R.E. (1990) Optimization of the annealing temperature for DNA amplification in vitro. *Nucleic Acids Res.* **18**, 6409–12.
11. Longo, M.C., Berninger, M.S. and Hartley, J.L. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* **93**, 125–8.

7. Related Products

Real-Time PCR Systems

Product	Size	Cat.#
GoTaq® Probe qPCR Master Mix	2ml	A6101
	10ml	A6102
GoTaq® Probe 1-Step RT-qPCR System	2ml	A6120
	12.5ml	A6121
GoTaq® Probe 2-Step RT-qPCR System	2ml	A6110
GoTaq® qPCR Master Mix (BRYT Green® Dye)	5ml	A6001
	25ml	A6002
GoTaq® 1-Step RT-qPCR System (BRYT Green® Dye)	5ml	A6020
GoTaq® 2-Step RT-qPCR System (BRYT Green® Dye)	5ml	A6010

Endpoint Hot-Start PCR

Product	Size	Cat.#
GoTaq® G2 Hot Start Polymerase	100u	M7401
	500u	M7405
	2,500u	M7406
	10,000u	M7408
GoTaq® G2 Hot Start Green Master Mix	100 reactions	M7422
	1,000 reactions	M7423
GoTaq® G2 Hot Start Colorless Master Mix	100 reactions	M7432
	1,000 reactions	M7433

Endpoint Standard PCR

Product	Size	Cat.#
GoTaq® G2 DNAPolymerase	100u	M7841
	500u	M7845
	2,500u	M7848
GoTaq® G2 Flexi DNAPolymerase	100u	M7801
	500u	M7805
	2,500u	M7806
	10,000u	M7808

7. Related Products (continued)

Endpoint Standard PCR (continued)

Product	Size	Cat.#
GoTaq® G2 Green Master Mix	100 reactions	M7822
	1,000 reactions	M7823
GoTaq® G2 Colorless Master Mix	100 reactions	M7832
	1,000 reactions	M7833
Pfu DNA Polymerase	100u	M7741
	500u	M7745

Reverse Transcription Enzymes and Systems

Product	Size	Cat.#
GoScript™ Reverse Transcription System	50 reactions (of 20µl)	A5000
	100 reactions (of 20µl)	A5001
GoScript™ Reverse Transcriptase	100 reactions (of 20µl)	A5003
	500 reactions (of 20µl)	A5004
GoScript™ Reverse Transcriptase Mix, Oligo(dT)	50 reactions (of 20µl)	A2790
	100 reactions (of 20µl)	A2791
GoScript™ Reverse Transcriptase Mix, Random Primers	50 reactions (of 20µl)	A2800
	100 reactions (of 20µl)	A2801
AccessQuick™ RT-PCR System	20 reactions	A1701
	100 reactions	A1702
	500 reactions	A1703
Access RT-PCR System	100 reactions	A1250
	500 reactions	A1280
Access RT-PCR Introductory System	20 reactions	A1260

BenchTop DNA Markers

Product	Size	Cat.#
BenchTop ΦX174 DNA/HaeIII Markers	250µl	G7511
BenchTop pGEM® DNA Markers	250µl	G7521
BenchTop PCR Markers	300µl	G7531
BenchTop 1kb DNA Ladder	600µl	G7541
BenchTop 100bp DNA Ladder	300µl	G8291

Molecular Weight Markers

Product	Size	Cat.#
1kb DNA Step Ladder	90µg	G6941
200bp DNA Step Ladder	100µg	G6961
100bp DNA Step Ladder	100µg	G6951
50bp DNA Step Ladder	90µg	G4521
25bp DNA Step Ladder	100µg	G4511
10bp DNA Step Ladder	32.5µg	G4471
1kb DNA Ladder	500µl	G5711

PCR Nucleotide Mix

Product	Size	Cat.#
PCR Nucleotide Mix, 10mM	200µl	C1141
	1,000µl	C1145
PCR Nucleotide Mix, 25mM	200µl	U1431
	1,000µl	U1432
dNTP Mix	200µl	U1511
	1,000µl	U1515
dNTP Sets (dATP, dCTP, dGTP and dTTP)	10µmol each	U1330
	25µmol each	U1420
	40µmol each	U1240
	200µmol each	U1410
dNTP Sets (dATP, dCTP, dGTP and dUTP)	10µmol each	U1335
	40µmol each	U1245

PCR Clean-Up System

Product	Size	Cat.#
Wizard® SV Gel and PCR Clean-Up System	50 preps	A9281
	250 preps	A9282

Additional size available

7. Related Products (continued)

PCR Cloning

Product	Size	Cat.#
pGEM®-T Easy Vector System I	20 reactions	A1360
pGEM®-T Easy Vector System II (includes JM109 Competent Cells)	20 reactions	A1380
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II (includes JM109 Competent Cells)	20 reactions	A3610

Staining Dye

Product	Size	Cat.#
Diamond™ Nucleic Acid Dye	500µl	H1181

8. Summary of Changes

The following changes were made to the 5/19 revision of this document:

1. The document design was updated.
2. Product and related product information were updated.

[a]Use of this product for basic PCR is outside of any valid US patents assigned to Hoffman La-Roche or Applera. This product can be used for basic PCR in research, commercial or diagnostic applications without any license or royalty fees.

[b]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.